OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and *Salmonella typhimurium*, is homologous to a family of bacterial regulatory proteins

(oxidative stress/autoregulation/LysR/NodD)

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ABSTRACT The oxyR gene is required for the induction of a regulon of hydrogen peroxide-inducible genes in Escherichia coli and Salmonella typhimurium. The E. coli oxyR gene has been cloned and sequenced, revealing an open reading frame (305 amino acids) that encodes a 34.4-kDa protein, which is produced in maxicells carrying the oxyR clone. The OxyR protein shows homology to a family of positive regulatory proteins including LysR in E. coli and NodD in Rhizobium. Like them, oxyR appears to be negatively autoregulated: an oxyR:: lacZ gene fusion produced 5-fold higher levels of β -galactosidase activity in oxyR null mutants compared to $oxyR^+$ controls, and extracts from an OxyR-overproducing strain were able to protect regions (-27 to + 21) of the oxyR promoter from DNase I digestion. DNA sequence analysis of the oxyR2 mutation, which causes overexpression of oxyR-regulated proteins in the absence of oxidative stress, showed that the oxyR2 phenotype is due to a missense mutation (C·G to T·A transition) that changes alanine to valine at amino acid position 234 of OxvR.

Cloning and DNA sequence analysis of the genes encoding bacterial and phage transcriptional regulatory proteins has facilitated the understanding of the molecular mechanisms used to control gene expression by directing attention to a few evolutionarily conserved domains in these regulatory proteins (1-4). Numerous transcriptional regulatory proteins contain a conserved "helix-turn-helix" domain by which many bacterial (and some eukaryotic) proteins recognize and bind to specific DNA sequences (reviewed in refs. 1 and 5). Several bacterial proteins have since been found to be alternative sigma factors for RNA polymerase (reviewed in ref. 2). Recently, it has also been noted that for many bacterial regulons, two proteins are involved in regulating gene expression and that these proteins share homology (reviewed in ref. 3). In these systems, one protein is thought to function as the sensor of an environmental change and the other component is a transcriptional activator. Through extensive sequence comparisons, Kofoid and Parkinson (4) have extended these classes and denoted transmitter and receiver domains in many bacterial regulatory proteins.

We have previously described a regulator, oxyR, of hydrogen peroxide-inducible genes in Salmonella typhimurium and Escherichia coli (6). Treatment of bacterial cells with low doses of hydrogen peroxide induces resistance to subsequent lethal doses of hydrogen peroxide and the synthesis of 30 proteins. Nine of these proteins, including catalase, glutathione reductase, and an alkyl hydroperoxide reductase, require the oxyR gene for hydrogen peroxide induction. Strains with deletions of oxyR are unable to induce this regulon and are hypersensitive to hydrogen peroxide. Strains carrying a dominant mutation, oxyR1 in S. typhimurium and oxyR2 in E. coli, are resistant to hydrogen peroxide killing and constitutively overproduce the oxyR-regulated proteins. As a first step toward understanding the molecular mechanism of OxyR action, we have sequenced the $oxyR^+$ and oxyR2 genes from E. coli and investigated the regulation of oxyR. Here we report that OxyR is a member of a family of

MATERIALS AND METHODS

bacterial regulator proteins.§

Plasmids. The inserts carried by pAQ16-pAQ21 are illustrated in Figs. 1 and 2A. pAQ16 and pAQ17 are derived from pUC12 (9). pAQ19 and pAQ21 carry inserts cloned into pUC18, and pAQ18 and pAQ20 carry inserts in pUC19. pAQ22 carries a 2.3-kilobase (kb) fragment isolated from TA4110 (*oxyR2*) chromosomal DNA digested with *Bam*HI and *Eco*RI and cloned into pUC18. pAQ23 carries a 0.2-kb *Hinc*II/*Ssp* I fragment of pAQ17 cloned into the unique *Sma* I site of pRS415 (10).

DNA Manipulations. DNA isolation, analysis, and cloning were generally carried out as described by Maniatis *et al.* (11). DNA sequencing was carried out by the dideoxynucleotide chain-termination method (12) with Klenow enzyme (Boehringer Mannheim) or Sequenase (United States Biochemical) enzyme. The C·G to T·A mutation at amino acid position 234 was generated by using an oligonucleotidedirected *in vitro* mutagenesis system from Amersham.

Maxicells. Plasmid-encoded proteins were labeled with $[^{35}S]$ methionine in maxicells as described by Sancar *et al.* (13). CSR603 (13) cells carrying the desired clones were grown to midlogarithmic phase ($A_{600} = 0.41-0.52$) in M9 medium containing 1% Casamino acids and thiamine (0.1 μ g/ml) and then irradiated 15 sec with a GE germicidal lamp at a 29.5-cm distance. After a 1-hr recovery, cells were treated with cycloserine (100 μ g/ml) and incubated in the dark for 8-12 more hr. The cells were then collected, resuspended in Hershey medium, and incubated for another hour, after which 5 μ l of [^{35}S]methionine (50 μ Ci; 1 Ci = 37 GBq) was added for 1 hr. Cells were collected, resuspended in Laemmli (14) buffer, and electrophoresed on a 12% polyacrylamide gel.

Primer Extension. Total cellular RNA was isolated from K12 and TA4112 by phenol extraction as described (15). A 30-nucleotide (CTCAAGATCACGAATATTCATTATC-CATCC) single-stranded end-labeled synthetic oligonucleotide was added to 25 μ g of RNA, was allowed to anneal, and then extended with reverse transcriptase (Life Sciences,

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[§]The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04553).

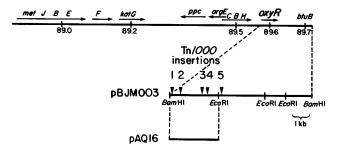


FIG. 1. Map position of oxyR in *E. coli*. The map position of oxyR relative to *metJBLF*, *katG*, *ppc*, *argECBH*, and *btuB* is based on the phenotype of Tn10-mediated deletions in *E. coli* and *S. typhimurium*. (The gene orientations were taken from refs. 7 and 8.) The lines below the chromosomal map denote the DNA carried by pBJM003 and the pAQ16 subclone.

Saint Petersburg, FL) as described (16). The extended products were analyzed by electrophoresis on 8% denaturing polyacrylamide gels. Dideoxynucleotide sequencing reactions primed from the end-labeled oligonucleotide were electrophoresed in neighboring lanes.

\beta-Galactosidase Assays. β -Galactosidase assays were performed as described by Miller (17) after cells were lysed as described (18).

Footprinting. $oxyR\Delta 3$ strains carrying pMC7 (carrying $lacI^{4}$; ref. 19) and pKK177-3 (carrying the *tac* promoter, derived from pKK223-3; ref. 20) or pAQ25 (oxyR with the *des* Shine-Dalgarno sequence cloned behind the *tac* promoter in pKK177-3; G.S., L. A. Tartaglia, and B.N.A., unpublished data) were treated with 1 mM isopropyl β -D-thiogalactopy-ranoside for 2 hr. Samples (5 ml) of the treated cells were then centrifuged and the pellets were resuspended in 800 μ l of 10 mM Tris-HCl (pH 7.5). The cells were lysed by two 10-sec sonication steps, centrifuged 5 min at 3000 × g to remove unlysed cells, followed by a second centrifugation at 13,000

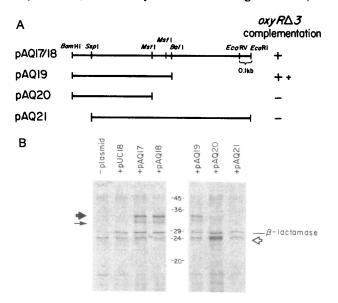


FIG. 2. Proteins encoded by oxyR subclones. (A) DNA carried by the oxyR subclones. pAQ17, pAQ18, and pAQ19, but not pAQ20 and pAQ21, are able to complement the hydrogen peroxide sensitivity of $oxyR\Delta3$. (pAQ17 and pAQ18 carry the same 1.5-kb fragment cloned in opposite orientations relative to the *lacZ* promoter in pUC12 and pUC19.) (B) Autoradiograph corresponding to the proteins produced by maxicells carrying various oxyR subclones. The large solid arrow points to the prominent 34-kDa protein encoded by pAQ17, pAQ18, and pAQ19. The open arrow denotes a prominent species <34 kDa encoded by pAQ20. (The small arrow indicates a minor 32-kDa species, possibly a modified form of OxyR, encoded by pAQ17, pAQ18, and pAQ19.)

× g to remove the membrane fraction. The footprinting reactions were carried out as described (21). Aliquots of the soluble fraction (final protein concentration, 5 or 50 μ g/ml) were incubated with a 200-base-pair (bp) BamHI/Tth111-I oxyR fragment (5 fM, labeled at the BamHI site with [γ^{-32} P]ATP) and poly(dI-dC) competitor DNA (0.1 μ g) for 10 min at room temperature, after which DNase I (2 μ l; 1.25 μ g/ml; Cooper Biomedical) was added for 1 min. The cleaved products were analyzed on an 8% denaturing polyacrylamide gel alongside a G/A sequencing ladder.

RESULTS

Cloning oxyR from E. coli. We previously isolated deletions of oxyR in S. typhimurium ($oxyR\Delta 2$, TA4108) and E. coli $(oxyR\Delta3, TA4112)$ by selecting for the loss of Tn10 insertions in the nearby argH (89.5 min) and btuB genes (89.7 min) (6). Since Tn10-mediated deletions extend in only one direction from the original insertion, oxyR had to be located in the region between argH and btuB as shown in Fig. 1. A 15.4-kb clone (pBJM003) carrying the E. coli btuB gene and a series pBJM003 derivatives carrying Tn1000 (γ - δ) insertions were described by Heller et al. (22). pBJM003 and five insertion derivatives were transformed into the E. coli oxyR deletion strain, and transformants were then tested for sensitivity to hydrogen peroxide and cumene hydroperoxide. The hypersensitivity to peroxides exhibited by $oxyR\Delta 3$ is corrected by pBJM003 and all of the Tn1000 derivatives except pBJM003-Tn1000-2 (data not shown). Therefore, the oxyR gene is located at or near the site of the Tn1000-2 insertion, about 800 bp from the BamHI site in pBJM003.

A 2.3-kb EcoRI/BamHI fragment of pBJM003, which carries the site of Tn1000-2 insertion, was isolated and cloned into the EcoRI and BamHI sites of pUC12 (pAQ16). This 2.3-kb insert complemented the peroxide hypersensitivity of $oxyR\Delta3$ (Table 1). To further define the sequences required for complementation of the chromosomal deletion of oxyR, a 1.0-kb internal EcoRV fragment was removed from the 2.3-kb clone to give pAQ17 and pAQ18. pAQ17 and pAQ18 were still able to complement the oxyR deletion strain (Table 1). The restriction map of pAQ17 is given in Fig. 2A, and the DNA carried by further subclones is indicated. The ability of the subclones to complement the $oxyR\Delta3$ sensitivity to hydrogen peroxide and cumene hydroperoxide is reflected by the zones of inhibition given in Table 1. pAQ19 still complements the $oxyR\Delta3$ sensitivity and even confers slightly greater resistance than any of the larger clones. pAQ20 and pAQ21 no longer complement $oxyR\Delta3$, indicating that essential portions of the oxyR gene have been deleted. pAQ16 and pAQ17 were also found to restore the inducibility of oxyRregulated proteins by hydrogen peroxide as determined by two-dimensional protein gels in oxyR deletion strains (data not shown).

Table 1.	Sensitivity t	o killing of	strains car	ying ox	yR clones
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		Zones of inhibition, mm		
		Hydrogen peroxide (10%)	Cumene hydroperoxide (5%)	
TA4467	$oxyR\Delta3/pUC18$	35	26	
TA4470	$oxyR\Delta3/pAQ16$	25	20	
TA4471	$oxyR\Delta3/pAQ17$	25	20	
TA4472	$oxyR\Delta3/pAQ18$	23	19	
TA4473	$oxyR\Delta3/pAQ19$	20	18	
TA4474	$oxyR\Delta3/pAQ20$	34	25	
TA4475	oxyR∆3/pAQ21	35	26	

The zones of inhibition were determined as described (6) except that the strains were grown in and plated on Luria broth (LB) containing ampicillin. The zone sizes in mm are the means of three determinations. **OxyR Is a 34-kDa Protein.** The proteins encoded by the various oxyR subclones were examined by labeling the plasmid-encoded proteins in maxicells. The vector control (pUC18) and pAQ17, -18, -19, -20, and -21 were transformed into CSR603, a $recA^-$, $uvrB^-$ strain. When this strain is irradiated with UV light, most of the chromosomal DNA is damaged, but, because of the large number of plasmids, some of these escape UV damage. The cells are unable to repair the UV damage and degrade all the damaged DNA. If [35 S]methionine is added several hours later, only proteins encoded by the undamaged plasmid DNA are synthesized and labeled. As shown in Fig. 2B, the clones capable of complementing the $oxyR\Delta3$ strain (pAQ17, -18, -19) encode a prominent

TTCACGTTGGCTTTAGTTATTCGAGTTGAGAAACTCTCGAAACGGGCAGTGACTTCAAG -35 -10 SD GATAATCGTTCATTGCTATTCTACCTATCGCCATGAACTATCGTGGCGATGGAGGATGG 90 Ser Gly Pro Leu His Ile Gly Leu Ile Pro Thr Val Gly Pro Tyr TCC GGA CCG CTG CAC ATT GGT TTG ATT CCC ACA GTT GGA CCG TAC 110 Leu Leu Pro His Ile Ile Pro Met Leu His Gln Thr Phe Pro Lys CTG CTA CCG CAT ATT ATC CCT ATG CTG CAC CAG ACC TTT CCA AAG 120 120 Leu Glu Met Tyr Leu His Glu Ala Gln Thr His Gln Leu Leu Ala CTG GAA ATG TAT CTG CAT GAA GCA CAG ACC CAC CAG TTA CTG GCG 140 Gln Leu Asp Ser Gly Lys Leu Asp Cys Val Ile Leu Ala Leu Val CAA CTG GAC AGC GGC AAA CTC GAT TGC GTG ATC CTC GCG CTG GTG 150 Lys Glu Ser Glu Arg Phe Ile Glu Val Pro Leu Phe Asp Glu Pro ARA GAG AGC GAA CGA TTC ATT GAA GTG CCG TTG TTT GAT GAG CCA 170 Met Leu Ala Ile Tyr Glu Asp His Pro Trp Ala Asn Arg Glu ATG TTG CTG GCT ATC TAT GAA GAT CAC CCG TGG GCG AAC CGC GAA 180 Cys Val Pro Met Ala Asp Leu Ala Gly Glu Lys Leu Leu Met Leu TGC GTA CCG ATG GCC GAT CTG GCA GGG GAA AAA CTG CTG ATG CTG 200 Glu Asp Gly His Cys Leu Arg Asp Gln Ala Met Gly Phe Cys Phe GAA GAT GGT CAC TGT TTG CGC GAT CAG GCA ATG GGT TTC TGT TTT 210 220 Glu Ala Gly Ala Asp Glu Asp Thr His Phe Arg Ala Thr Ser Leu GAA GCC GGG GCG GAT GAA GAT ACA CAC TTC CGC GCG ACC AGC CTG 230

 Glu Thr Leu Arg Asn Met Val Ala Ala Gly Ser Gly Ile Thr Leu

 GAA ACT CTG CGC ACA ATG GTG GCG GCA GGT AGC GGG ATC ACT TTA

 240
 MSt I

 250

 Leu Pro Ala Leu Ala Val Pro Pro Glu Arg Lys Arg Asp Gly Val

 CTG CCA GCG GCT GTG CCG CCG GAG CGC AAA CGC GAT GGG GTT

 260

 Val Tyr Leu Pro Cys Ile Lys Pro Glu Pro Arg Arg Thr Ile Gly

 GTT TAT CTG CCG TGC ATT AAG CCG GAA CCA CGC CGC ACT ATT GGC

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Translated Mol. Weight = 34366.46

FIG. 3. Sequence of oxyR. The start of the major oxyR transcript is denoted by the arrow. The region of the oxyR promoter protected against DNase I digestion by extracts from OxyR-overproducing cells is indicated by shading. SD, Shine-Dalgarno sequence.

34-kDa protein and a minor 32-kDa protein that are not present in the pUC18 vector control lane. The cells carrying pAQ20 encode a protein that is smaller than 34 kDa, possibly a truncated form of the major 34-kDa species, suggesting that the *Mst* I end of the clone corresponds to the C-terminal end of the OxyR protein. pAQ21 does not encode any plasmidspecific proteins other than the β -lactamase, suggesting that the *Bam*HI to *Ssp* I section of the *oxyR* clone may carry the *oxyR* promoter region.

The Sequence of oxyR Reveals Homology to a Family of Transcriptional Activator Proteins in Bacteria. The DNA sequence of a 1200-bp region extending from the BamHI site toward the EcoRI site was determined. The one long open reading frame encoded by the DNA sequence corresponds to a 34.4-kDa protein extending from the BamHI site toward the EcoRI end (Fig. 3). The size and orientation of the protein agree with the maxicell data. The predicted charge of the OxyR protein (pI 6.18-6.9) agrees with the position of the protein on two-dimensional gels probed with antibodies directed against a β -galactosidase–OxyR fusion protein (G.S. and B.N.A., unpublished results), and the codon usage is typical of E. coli (23). To confirm that the reading frame was correct, we created a frameshift mutation by filling-in the unique Tth111-I site within oxyR (a net two-nucleotide insertion). The frameshift mutant was unable to complement the hydrogen peroxide sensitivity of an oxyR deletion (data not shown).

Comparing the amino acid sequence of the OxyR protein with sequences in the Dayhoff data bank, we found that OxyR shows extensive homology to the E. coli protein LysR (Fig. 4). LysR is a positive regulator of lysA, which encodes the enzyme responsible for the last step in lysine biosynthesis (24, 27). OxyR also shares significant homology with the Rhizobium meliloti 1021 protein NodD (25) (Fig. 4) and other Rhizobium NodD proteins (28). NodD regulates several genes involved in the formation of nodules in the symbiotic relationship between Rhizobium and legumes (25, 28). Henikoff et al. (29) have recently recognized that these proteins and others [E. coli IlvY, regulator of ilvC and other genes in the isoleucine-valine biosynthetic pathway (30); E. coli and S. typhimurium CysB, positive regulator of the cysteine regulon (31); S. typhimurium MetR, regulator of the metE and metH genes (32); and the Enterobacter cloacae ampR regulator of the ampC gene, which confers resistance to cephalosporin (33)] comprise a family of bacterial regulatory

OxyR 1 LysR 1 NodD 1	MNIRDLEYLVA-LAEHR-H-FRRAADSCHVSQPTLSGQIRK MAAVNERHIEIFHAVMTAGSLTEAAHLLHTSQPTVSRELAR MPHMRFRGLDLNLLVALDALMTERKLTAAARRINLSQPAMSAAIAR
39 42 47	LEDELGYMLLERTSRKVLFTQAGMLLVDQARTVLREVKVLKEMASQ FEKVIGLKLFERVRGRLHPTVQGLRLFEEVQRSWYGLDRIVSAAES LRTYFGDELFSMQGRELIPTPRAEALAPAVRDALLHIQLSVIAWDP
85 88 93	QGETMSGPLHIGLIPTVGPYLLP-HIIPMLHQTFPKLEMYLHE- LREFRQGELSIACLPVFSQSFLPQLLQPFLAR-YPDVSLNIVPQES LNPAQSDRRFRIILSDFMILVFFARIVERVAREAPGVSFELLP-LD
127 133 138	PLLEEWLSAORHDLGL-TETLHTPAGTER-TELLSLDEVCVLPP
172 175 181	GHPLAVKKVLTPDDFQGENYISLSRTDSYRQLLDQLFTEHQVKRRM
218 221 225	IVETHSAASVCAMVRAGVGISVVNPLTALDYAASGLVVRRFSIAVP
263 267 268	

FIG. 4. Homology among OxyR, LysR, and NodD. Shading between residues indicates identity or conservative leucine-valineisoleucine substitutions between OxyR and LysR (24) and NodD (25). Inducer-independent missense mutations in OxyR and NodD (26) are indicated with arrows. Amino acids are designated by the single-letter code. proteins. The sequence of *Pseudomonas aeruginosa trpI*, the positive regulator of the *trpAB* operon, has shown that TrpI is yet another member of the family (34). All of the bacterial regulatory proteins described above are between 30 and 35 kDa in size and act as transcriptional activators. The most extensive homology is in the N-terminal ends of the proteins, although there is homology throughout the amino acid sequences.

Start of the oxyR Transcript. Total cellular RNA was isolated from wild-type and oxyR2 mutant cells. The start point of the oxyR transcript was determined by extension of a primer hybridizing to the 5' region of the oxyR mRNA (Fig. 5). The major transcript starts with a G residue. Corresponding -10 and -35 sequences that contain five of the six residues found in the canonical sigma70 promoters are indicated in Fig. 3. The similar levels of the oxyR transcript in both the wild-type and oxyR2 mutant strains indicated by the approximately equal intensity of the primer-extension products suggests that the constitutive phenotype of the oxyR message and protein.

The Constitutive Phenotype of the oxyR2 Mutant Is Due to an Alanine to Valine Missense Mutation. An oxyR2 mutant strain constitutively overexpresses oxyR-regulated catalase and alkyl hydroperoxide reductase activities (6). To learn more about the mechanism of oxyR activation, the mutant oxyR2 gene was cloned. Chromosomal DNA was isolated from the oxyR2 mutant and digested with EcoRI and BamHI. Fragments between 2.0 and 2.3 kb were cloned into the EcoRI and BamHI sites of pUC18. Colonies obtained after transforming $oxyR\Delta3$ were screened for hybridization to a fragment of the wild-type oxyR clone. Three oxyR2 clones were isolated in this fashion, of which pAQ22 is one representative. Although $oxyR\Delta3$ carrying pAQ22 (TA4476) was very slow growing and pAQ22 was unstable, the strain had increased levels of the oxyR-regulated proteins and increased resistance to hydrogen peroxide (data not shown).

We sequenced the entire oxyR2 coding region and found that the only difference between oxyR2 and the wild-type sequence was a C·G to T·A missense mutation causing a valine to be substituted for an alanine at amino acid position 234. To confirm that the C·G to T·A missense mutation was in fact the sole mutation to cause the oxyR2 constitutive phenotype, this transition mutation was also generated by site-specific mutagenesis. The site-specific mutant contained on pUC18 conferred the same phenotypes as pAQ22, showing that the valine for alanine substitution at position 234 in OxyR is sufficient to cause constitutive induction of hydrogen peroxide-inducible genes.

oxyR Is Negatively Autoregulated by the Binding of OxyR to Its Own Promoter. To study the expression from the oxyR promoter, the oxyR upstream and promoter region sequences were fused to the *lacZ* gene on a multicopy vector (pRS415) to give pAQ23. This construct was moved into oxyR⁺ (TA4477) and oxyR Δ 3 (TA4478) backgrounds, and the level of β -galactosidase activity was determined during exponential growth. Interestingly, the oxyR::*lacZ* fusion gave 5-fold

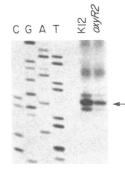


FIG. 5. Start of the *oxyR* transcript. The start of the major *oxyR* transcript in wild-type and *oxyR2* mutant cells was determined by primer-extension studies. Occasional double bands in the adjacent sequencing ladder are due to hybridization between the 30-base primer and M13.

higher levels of activity in the oxyR deletion background (19,000 units; $A_{600} = 0.593$) compared to the $oxyR^+$ background (3800 units; $A_{600} = 0.613$). This finding suggests that OxyR is a negative regulator of its own expression.

To learn more about the nature of the repression we investigated the ability of the OxyR protein to bind its own promoter. Extracts made from cells that greatly overproduce the OxyR protein were added to oxyR promoter fragments and then tested for the ability to protect against DNase I digestion. A distinct region of protection was seen extending from -27 to +21 relative to the start of the oxyR transcript (Fig. 6). This protection is not seen with extracts made from strains that do not overproduce OxyR and suggests that the mechanism by which OxyR negatively autoregulates its expression is by binding to, and therefore blocking, the DNA sequences that are required for its own transcription.

DISCUSSION

The cloning and DNA sequence of the *E. coli oxyR* gene, a positive regulator of a regulon for defenses against oxidative stress, revealed that OxyR is a 34-kDa protein that shows significant homology to a family of bacterial regulatory proteins. All of these regulators are between 30 and 35 kDa in size, all act as positive regulators, and many are known to

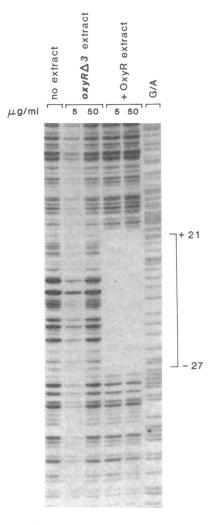


FIG. 6. Footprint of OxyR on the oxyR promoter. A 5'end-labeled oxyR promoter fragment was incubated with extracts from an oxyR deletion strain or an OxyR-overproducing strain (final concentration, 5 and 50 μ g of protein per ml) and then digested with DNase I as described. The protection is shown for the noncoding strand. negatively regulate their own expression (29). The role of oxyR as a positive regulator has been described (6). The increased expression from a heterologous oxyR::lacZ gene fusion in an oxyR deletion strain compared to an $oxyR^+$ strain suggests that OxyR is also a negative regulator of its own expression. Interestingly, while OxyR, LysR, NodD, IlvY, and MetR all negatively regulate their own genes, they positively regulate a transcript from an overlapping promoter on the complementary strand (29, 36). The oxyR system differs from the other systems in that the transcript made opposite the oxyR structural gene is very small (only 100 nucleotides) and probably does not encode a protein (G.S. and B.N.A., unpublished data).

The footprinting experiments described here suggest that the OxyR protein binds to DNA. Purified OxyR can also protect specific regions of other oxyR-regulated promoters from DNase I digestion (G.S., L. A. Tartaglia, and B.N.A., unpublished data). Based on sequence analysis, Henikoff *et al.* (29) suggest that the LysR class of bacterial regulators have helix-turn-helix motifs toward the N-terminal domains of the proteins, but proof that these helices are interacting with the DNA will require structural studies. Another member of the LysR class, NodD, has been shown to bind a defined DNA sequence (NOD box; ref. 35), and studies on chimeric NodD proteins with C-terminal domains from different strains of *Rhizobium* do suggest that the N-terminal portion of the protein is involved in DNA recognition (28).

The inducer-responsive domain may be near the C terminus. Our finding that the mutation causing the constitutive phenotype maps within this region supports this claim. An inducer-independent *nodD* mutation was also found to map in the C-terminal region of NodD (ref. 26; also indicated in Fig. 4). It was surprising that the constitutive oxyR2 phenotype was solely due to an alanine to valine missense mutation, but it has been found that an inducer-independent *crp* mutation can also be caused by an alanine to valine change (Sankar Adhya, personal communication). Possibly, the slightly larger size of the valine compared to the alanine causes a significant conformational change.

The molecular mechanism by which the OxyR protein is activated by oxidative stress is of interest. The fact that the constitutive phenotype of the oxyR2 mutant is due to a missense mutation in OxyR and not due to overproduction of oxyR mRNA suggests that the OxyR protein itself may be modified to induce the regulon under stress conditions. It may be significant that none of the six cysteine residues found in the OxyR protein is present in LysR or NodD since cysteines might easily be oxidized. We are using the cloned oxyR gene to overproduce OxyR and to allow purification of the protein to determine the exact manner of OxyR activation by oxidative stress.

Note Added in Proof. We have recently learned that OxyR corresponds to the *mom* gene repressor (MomR), the methylationdependent regulator of the *mom* gene of phage Mu (M. Bölker and R. Kahmann, personal communication).

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